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Studies of Waterborne Agents of Viral Gastroenteritis

Vermont Univ., Burlington. Coll. of Medicine

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

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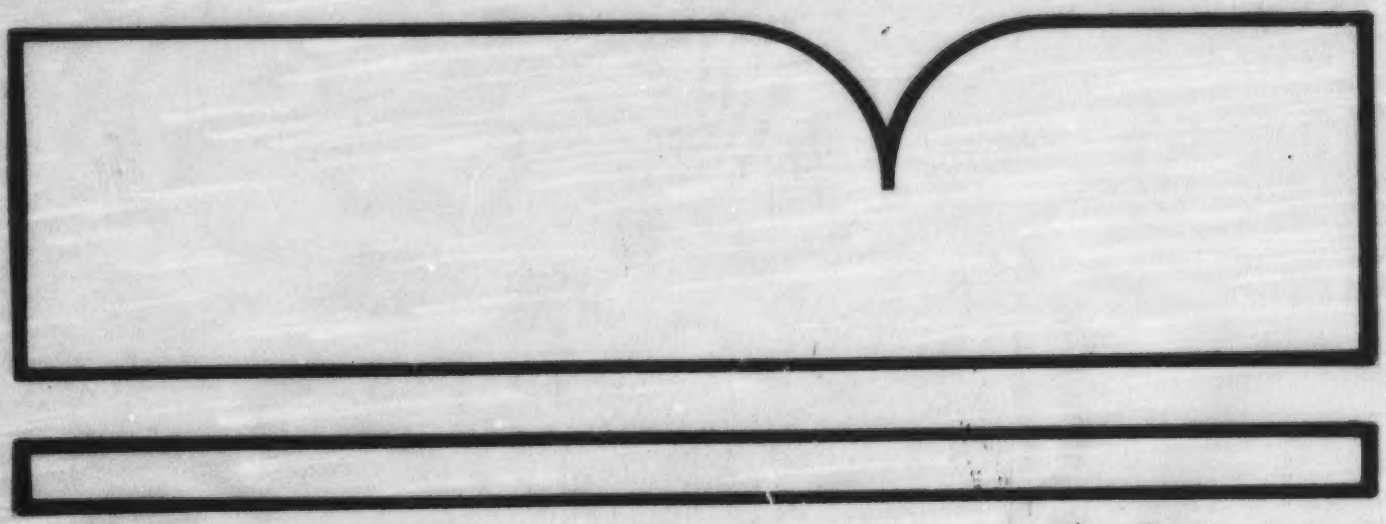

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STUDIES OF WATERBORNE AGENTS OF VIRAL GASTROENTERITIS

by

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Grant No. R806546

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OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
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16. ABSTRACT

The etiologic agent of a large outbreak of waterborne viral gastroenteritis was detected employing immune electron microscopy (IEM) and a newly developed solid phase radioimmunoassay (RIA). This agent, referred to as the Snow Mountain Agent (SMA), is 27-32 nm. in diameter, has cubic symmetry, and is morphologically similar to, but antigentically distinct from the previously described Norwalk and Hawaii agents. After an incubation period of 18 to 48 hours, the agent is shed in stools of acutely ill individuals for a period of one to five days. Employing both IEM and RIA, serum antibody rises were observed in 3/3 naturally occurring cases and in 8/9 cases of experimentally-induced illness in normal volunteers (the studies in normal volunteers had been carried out prior to the initiation of studies supported by this grant). IEM and RIA appeared to be equally sensitive for detection of antibody rises, but the RIA was more sensitive than IEM for the detection of SMA in stool specimens. Preliminary attempts to cultivate the agent in vitro were unsuccessful.

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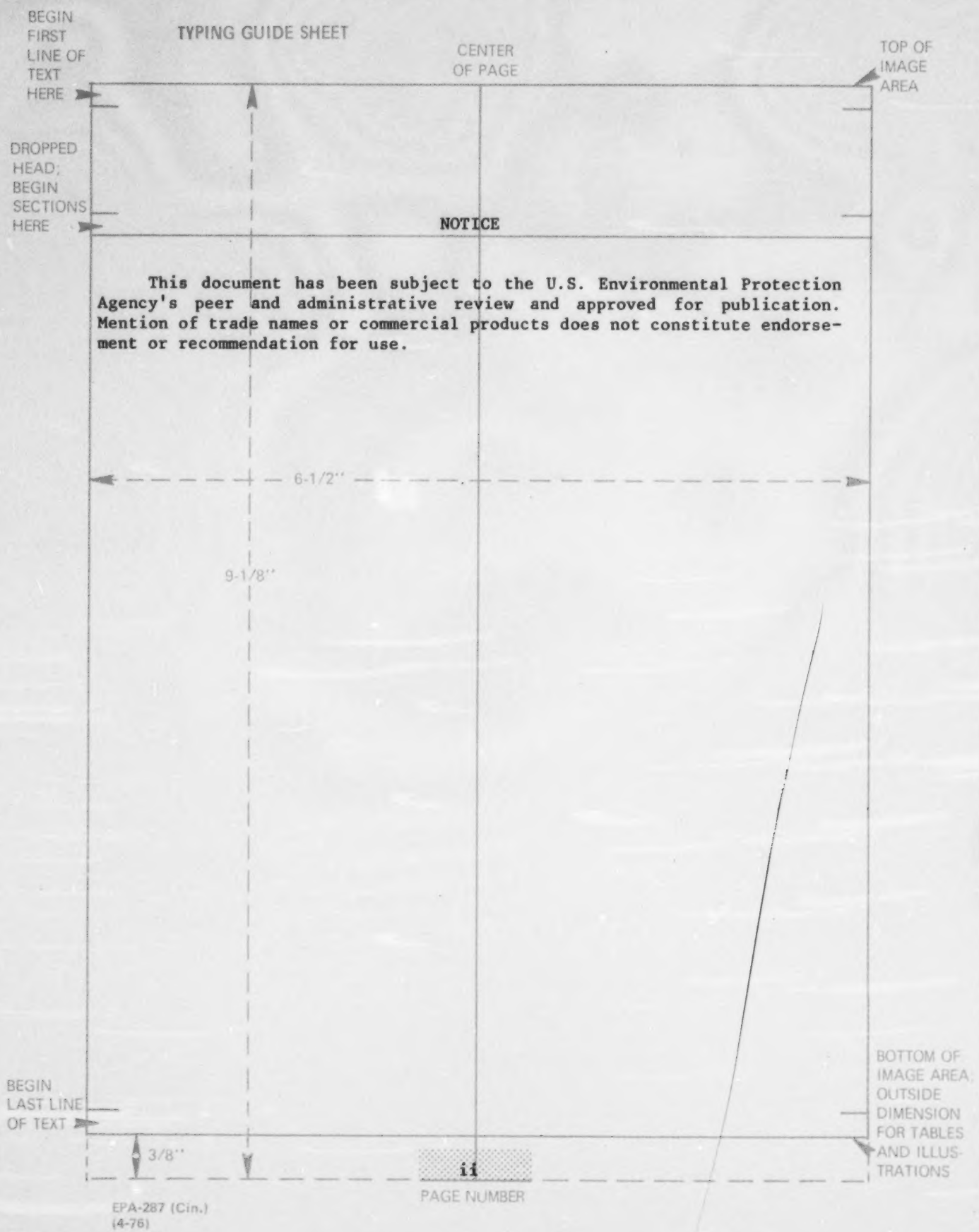
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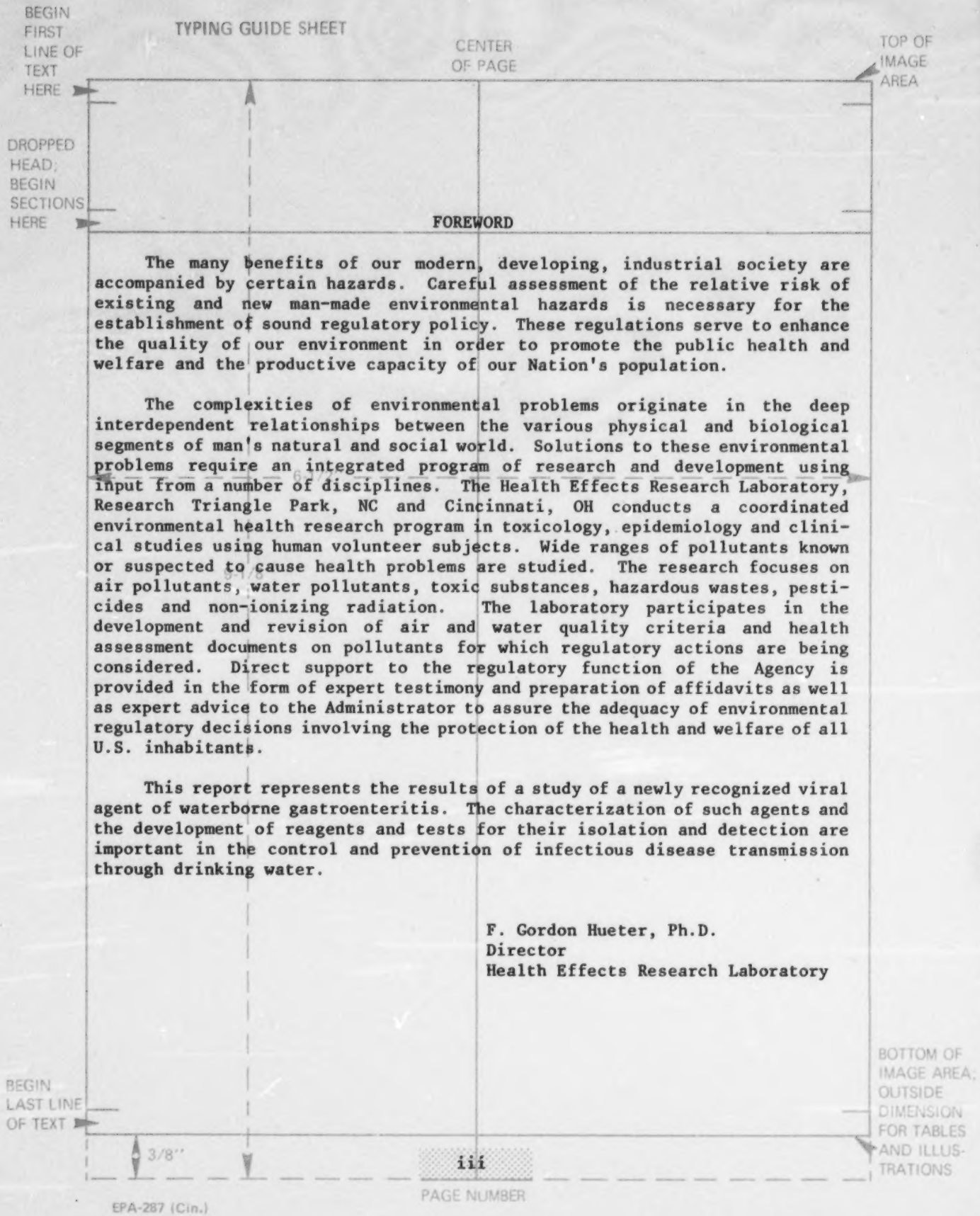
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FOREWORD
The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.
The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH conducts a coordinated environmental health research program in toxicology, epidemiology and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides and non-ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.
This report represents the results of a study of a newly recognized viral agent of waterborne gastroenteritis. The characterization of such agents and the development of reagents and tests for their isolation and detection are important in the control and prevention of infectious disease transmission through drinking water.
F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory
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ABSTRACT

The etiologic agent of a large outbreak of waterborne viral gastroenteritis was detected employing immune electron microscopy (IEM) and a newly developed solid phase radioimmunoassay (RIA). This agent, referred to as the Snow Mountain Agent (SMA), is 27-32 nm. in diameter, has cubic symmetry, and is morphologically similar to, but antigenically distinct from the previously described Norwalk and Hawaii agents. After an incubation period of 18 to 48 hours, the agent is shed in stools of acutely ill individuals for a period of one to five days. Employing both IEM and RIA, serum antibody rises were observed in 3/3 naturally occurring cases and in 8/9 cases of experimentally-induced illness in normal volunteers (the studies in normal volunteers had been carried out prior to the initiation of studies supported by this grant). IEM and RIA appeared to be equally sensitive for detection of antibody rises, but the RIA was more sensitive than IEM for the detection of SMA in stool specimens. Preliminary attempts to cultivate the agent in vitro were unsuccessful.

The development of the RIA for SMA provides a sensitive and efficient method with which to assess the epidemiologic significance of this agent and with which to begin evaluation of potential control measures.

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SECTION 1

INTRODUCTION

Acute viral gastroenteritis represents a significant source of morbidity in the United States and throughout the world. In the United States, approximately 25 days are lost from work or school per 100 individuals per year as a result of viral gastroenteritis. As such, it has been estimated to be the second most common disease experience among American families, surpassed in frequency only by acute viral respiratory tract infections (1). In addition, acute viral gastroenteritis appears to be a major contributor to infant mortality in developing countries (2). The development of effective control measures has been hindered by the lack of information concerning the specific etiology of presumed cases of "viral gastroenteritis," as well as by the poor understanding of the transmission and epidemiology of this disease. Recently, considerable new information has emerged in this area, including the description of the "Norwalk-like agents" which likely account for a significant portion of viral gastroenteritis (3). A recently detected member of the above group of agents is the Snow Mountain Agent or "SMA", which was described by the principal investigator in 1979 and is the subject of the studies carried out during this project period (4). These studies have demonstrated that the Snow Mountain Agent is the etiologic agent of a large waterborne outbreak of gastroenteritis. Evidence suggests that the outbreak may have been associated with improperly maintained water supplies (4).

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Since the precise mode of transmission and spread of other Norwalk-like agents is largely unknown, it is uncertain what proportion of acute viral gastroenteritis is a reflection of such waterborne spread. Studies of potential environmental sources of infection of Norwalk-like agents have been hampered by the absence of suitable methods of detection, including *in vitro* cultivation systems. A waterborne source of infection is suspected in the original outbreak in Norwalk, Ohio but no common source was detected (5). The Norwalk agent has subsequently been associated with three separate waterborne outbreaks of gastroenteritis in the United States during 1978, involving 937 individuals(6). A massive waterborne outbreak in Australia, in which more than 2,000 individuals were affected, has also been associated with the Norwalk agent (7). Thus, waterborne spread is clearly a means of transmission for Norwalk-like agents although how often such waterborne spread occurs is not known at present.

Data concerning the overall impact of waterborne disease in the United States are fragmentary, but the most recent CDC summary indicates that the total number of cases associated with reported outbreaks in 1978 numbered 11,435, which is a three fold increase from those reported in 1977 (6). Both of these figures likely represent gross under-reporting of the problem. Of particular interest is the fact that no etiology has been established in more than 50% of outbreaks of gastroenteritis reported to the CDC during 1978, despite intensive analysis of samples provided. Of outbreaks in which an etiologic agent was determined, the Norwalk agent accounted for approximately 20% (6). Since the radioimmunoassay for the Norwalk agent does not detect antigenically distinct agents such as SMA, it is conceivable that the Norwalk-like agents as a group may account for

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a much greater proportion of water related illness. It should be also noted that in contrast to several other waterborne pathogens, the Norwalk-like agents undergo rapid person-to-person spread once infection has occurred, so that the impact of waterborne transmission may be multiplied many fold.

The development of sensitive and efficient methods for detection of waterborne agents of viral gastroenteritis such as the Snow Mountain Agent, represents the first step in the development of potentially effective control measures. Because of the likely association of these agents with waterborne spread, the development of effective methods of decontamination of water sources may represent an important control measure for this disease.

SECTION 2

CONCLUSIONS

The etiologic agent of a major waterborne outbreak of acute gastroenteritis is a virus which will now be referred to as the Snow Mountain Agent (SMA) (4). This agent is 27 to 32 nm in size, has cubic symmetry, and is morphologically indistinguishable from the Norwalk and Hawaii agents. SMA, however, appears to be antigenically distinct from the Norwalk and Hawaii agents.

Sensitive in vitro tests have been developed for the detection of SMA in stools from both naturally-occurring and experimentally-induced illness, and for the detection of specific antibody directed against the agent. The first in vitro test which was developed was that of immune electron microscopy (IEM), in which the agent is aggregated in stool specimens by use of specific antibody. Conversely, the stool specimen is employed as a source of antigen with which to detect antibody in serum specimens from individuals undergoing infection. Although immune electron microscopy is a important technique, it is too cumbersome for the examination of large numbers of specimens. Therefore, major emphasis was placed on the development of a sensitive radioimmunoassay. The solid phase radioimmunoassay which was developed for SMA is highly sensitive in the detection of antigen in stool specimens as well as anti-SMA antibody in sera. RIA appeared to be equally as sensitive as IEM in the detection of serum antibody rises, but appeared considerably more sensitive than IEM in the detection of virus particles and/or antigen in stool filtrates.

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Studies employing both immune electron microscopy and radioimmunoassay demonstrated that virus is shed 24-48 hours after challenge, largely during the height of illness, and for up to five days post challenge. Serum antibody rises can be detected regularly three weeks after challenge.

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The radioimmunoassay should provide a powerful technique with which to conduct further studies of the Snow Mountain Agent.

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SECTION 3

RECOMMENDATIONS

The development of a radioimmunoassay for the Snow Mountain Agent represents a major advance in this field. This powerful technique will enable assessment of the relative significance of SMA in acute gastroenteritis to be carried out, particularly in the setting of waterborne outbreaks. We would recommend that the following studies, which can now be conducted efficiently, be carried out:

1) Assessment of the role (frequency) of infection with SMA in sporadic and epidemic forms of acute viral gastroenteritis. Primary emphasis should be placed on those outbreaks in which a waterborne or common source has been identified.

2) Evaluation of the seroepidemiology of infection with Snow Mountain Agent, employing age, socioeconomic, and geographically distributed populations.

3) Sampling of environmental sources, particularly water supplies, should be carried out. This can be done on a pilot project basis employing the radioimmunoassay developed in our laboratory. However, extensive sampling will not be practical until the development of suitable <u>in vitro</u> cultivation systems is accomplished.

4) Because relatively little work has been conducted in the development of <u>in vitro</u> cultivation systems, a major effort should be expended

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on the establishment of tissue and/or organ culture systems with which to cultivate these viruses. The availability of the radioimmunoassay should facilitate the search for such systems.

5) Because of the complex and highly specialized nature of the radioimmunoassay, efforts should be carried out to develop stable enzyme-linked immunosorbent reagents with which to carry out these assays. The development of such tertiary tests will likely depend on highly specific immunologic reagents generated either in animals or through monoclonal antibody production. Generation of the latter will also depend on the development of highly purified antigens either directly from stool filtrates or more likely, following <u>in vitro</u> cultivation of the agents.

6) Because of the documented waterborne spread of viral agents such as SMA, the assessment of control measures involving decontamination of water supplies should be carried out. Radioimmunoassays can be employed in such evaluations, although infectivity in either <u>in vitro</u> cultivation systems or animal models may be a more significant endpoint.

7) Methods which have been successfully applied to the study of the Snow Mountain Agent, should also be applied to other non-cultivatable agents of viral gastroenteritis for which no suitable tertiary assay exists.

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SECTION 4

MATERIALS AND METHODS

The stool and serum specimens for this study were derived from an outbreak of acute gastroenteritis at a resort (Snow Mountain) near Granby, Colorado, in December, 1976 which has been subsequently reported by the principal investigator (Lancet i:964, 1979). The characteristics of illness were typical of those of acute viral gastroenteritis, namely fever, vomiting, and diarrhea which lasted 24 to 48 hours. The attack rate was high (418 of 762 individuals at risk), and person-to-person spread was noted. Illness was associated with consumption of water or ice-containing beverages in a dose response manner ($p < .0001$). Stool specimens were negative for conventional bacterial pathogens, and virus cultures employing standard tissue culture systems were similarly negative. A limited amount of material (stool and sera) was available to the principal investigator from the naturally-occurring outbreak. Examination of five stool specimens from acutely ill cases by immune electron microscopy (see below) by the principal investigator revealed virus-like particles, 27 to 32 nm in diameter, morphologically indistinguishable from the Norwalk agent, in two specimens. Because of the limited amount of material available from the naturally-occurring outbreak, the principal investigator (previous to this study) had performed a series of studies in normal volunteers to determine infectivity of the preparations and to generate additional reagents for <u>in vitro</u> studies. Stool preparations

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which contained the above described particles were prepared as 2% suspensions in veal infusion broth and subjected to conventional safety tests (8). One cc of the above filtrate was administered orally to two normal volunteers who subsequently developed vomiting and diarrhea. Ten additional volunteers had been challenged with various dosage of infectious inoculum, and illness had been induced in seven of them. Incubation periods of illness were approximately 33 hours, and disease manifestations lasted 24 to 48 hours. All stools passed by volunteers, along with 30 cc of serum obtained prior to and three weeks after challenge, have been saved and stored at -70C. These stools and sera, along with the material available from the naturally-occurring outbreak, represent the reagents which have been used in the studies described below.

To perform the studies to be described, stool filtrates are prepared as 2% suspensions in veal infusion broth supplemented with 0.5% bovine serum albumin. After low speed centrifugation, the suspensions are filtered through nitrocellulose filters of decreasing pore size to a final filtration through filters of .45 mu in size. Currently 67 such filtrates have been prepared from material derived from the Snow Mountain outbreak and subsequent volunteer studies.

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SECTION 5

EXPERIMENTAL PROCEDURES

IMMUNE ELECTRON MICROSCOPY (IEM).

The initial <u>in vitro</u> technique employed to detect the Snow Mountain Agent (SMA) and antibody to SMA is immune electron microscopy. The specific methods employed are as follows:

(1) a 2% stool filtrate is prepared in veal infusion broth with 0.5% bovine serum albumin.

(2) 0.8 cc of the stool filtrate is reacted with 0.2 cc of convalescent serum overnight at 4°C.

(3) the reaction mixture is centrifuged at 17,000 rpm for 90' in an RC2B Sorvall centrifuge with a fixed angle rotor (SS34).

(4) the supernatant is discarded and the pellet is resuspended in 1 drop of distilled water.

(5) the suspension is placed on a 400 mesh Formvar grid, stained with 2% phosphotungstic acid, and examined under a Phillips 300 electron microscope.

(6) the agent is detected as individual particles or aggregates coated with antibody. Conversely, employing a filtrate which contains a known concentration of antigen, antibody content in serum specimens can be assayed in a semi-quantitative manner, on a scale of 0 to 4+.

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RADIOIMMUNOASSAY (RIA) for SMA ANTIGEN.

The RIA for SMA antigen as currently employed in our laboratory is performed as follows:

(1) purified anti-SMA IgG is prepared by $(NH_4)_2\ SO_4$ precipitation of convalescent (post-challenge) serum and dialysis with .005 M phosphate buffered saline (pH 8) at 4°C for 5 days. The globulins are purified by passage through a DEAE cellulose ion exchange column (Whatman DE52) at pH 8.0 with .005 M PBS.

(2) the purified IgG is labeled with ^{125}I by chloramine-T reaction.

(3) wells in polyvinyl microtiter plates are coated with a 1:10,000 dilution of either pre- or post-challenge serum overnight at room temperature.

(4) after washing out the wells, varying dilutions of stool filtrates are incubated in each well overnight at room temperature.

(5) after additional washing, 50 ul of purified IgG containing 200,000 cpm of ^{125}I are added to each well, and incubated for 4 hours at 37°C.

(6) the plates are washed again, and individual wells are counted in a gamma counting system. Differences in binding of greater than 2 (P/N 2) when wells coated with pre- and post-serum are compared, indicate the presence of antigen.

RIA FOR ANTIBODY TO SMA.

The methods are as follows:

(1) wells in polyvinyl microtiter plates are coated with a 1:10,000 dilution of a post-challenge serum specimen derived from a case of SMA-induced illness.

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(2) 25 ul of a standard stool preparation containing SMA is added to the wells, incubated overnight at room temperature, and subsequently washed. This stool preparation results in a P/N 3 in the above assay under standard conditions.

(3) ten-fold dilutions of the serum to be tested, in 40 ul aliquots, are added to each well and again incubated overnight at room temperature.

(4) 10 ul of ^{125}I labeled anti-SMA IgG is added to each well and incubated for 4 hours at 37°C.

(5) the plates are washed and counted as above. The titer of serum obtained is the reciprocal of the highest dilution which results in 50% or greater reduction in counts when compared to a PBS control.

IN VITRO CULTURE TECHNIQUES

(1) Inoculation of tissue cultures

(a) Tissue cultures of human diploid fibroblasts, human embryonic kidney and human hepatocytes were grown to semi-confluent states in 48 well microtiter plates. Aliquots (0.05ml) of stool filtrates known to contain SMA were inoculated. The inoculum was incubated for 2 hours at 33°C, and fresh minimal essential medium with 2% fetal calf serum wass added. Cultures were incubated at 33° and 37°C. Cultures were maintained for 2 weeks and then underwent two blind passages.

(b) Centrifugation of inoculum

The above tissue culture preparations were also grown in 1 dram vials, and 0.1ml of SMA containing inocula

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was inoculated by centrifuging at 1200 x g for 1 hour at 25°C in a swinging bucket rotor. Culture was subsequently carried out as described above.

(c) Inoculation of human mononuclear cells

Human circulating mononuclear cells were obtained from heparinized venous blood after hypaque-ficoll gradient centrifugation as previously described (10). Mononuclear cells were inoculated with 0.1ml of SMA containing inoculum, at concentrations of 10^0 to 10^{-6}. Mononuclear cell cultures were maintained in RPMI 1640 with 10% fetal calf serum.

(d) Detection system for SMA

The presence of SMA was sought by examination of tissue cultures for the development of cytopathic effect. Supernatants from tissue and mononuclear cell cultures were examined for the presence of SMA by immune electron microscopy.

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SECTION 6

RESULTS AND DISCUSSION

DETECTION OF SNOW MOUNTAIN AGENT BY IMMUNE ELECTRON MICROSCOPY.

Examination of stool filtrates from five of the naturally-occurring cases from the Snow Mountain outbreak revealed 27 to 32 nm virus-like particles in two of the specimens. Examination of stools from the nine cases of experimentally-induced illness revealed similar virus-like particles in stools from three of these cases. Particles were seen most frequently 24 to 48 hours after the onset of illness, and were not detected more than 72 hours after illness had begun. All stools were examined, and the number of particles was not associated with changes in stool characteristics. Particles appeared to have cubic symmetry with occasional groups of empty particles. The morphology of these particles was similar to that previously described for the Norwalk and Hawaii agents. Of interest, a number of stool filtrates contained 22 to 25 nm particles, often with large groups of empty particles. These smaller particles were seen both in association with the larger particles and occasionally by themselves, both in stools which contained SMA and in those which did not. The smaller particles were seen sporadically 24 to 96 hours after onset of illness. As will be discussed below, serum antibody rises were directed only at the larger particle and not at the smaller one. This observation is consistent with the interpretation that the larger particle is the etiologic agent of Snow Mountain outbreak. The significance of the smaller particle is not known at present.

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DETECTION OF ANTIBODY TO SNOW MOUNTAIN AGENT BY IMMUNE ELECTRON MICROSCOPY.

The results of antibody determinations and serum specimens from subjects challenged with the Snow Mountain Agent as determined by immune electron microscopy are presented in Table 1. Eight of nine volunteers with experimentally-induced illness demonstrated serum antibody rises to the 27 to 32 nm particle, along with three of three naturally-occurring cases (Nos. 11, 12 and 13) which were tested. None of three volunteers who were challenged with the Snow Mountain Agent but did not become ill manifested serum antibody rises.

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TABLE 1. ANTIBODY RATINGS IN SERUM SPECIMENS FROM SUBJECTS CHALLENGED WITH SNOW MOUNTAIN AGENT (SMA) AS DETERMINED BY IMMUNE ELECTRON MICROSCOPY+

Subject	Illness Following Challenge	Pre-challenge Serum	Post-challenge Serum#
1	yes	< 1	4+
2	yes	2+	3+
3	yes	< 1	2+
4	no	3+	3+
5	yes	< 1	3+
6	yes	< 1	2+
7	yes	1+	1+
8	yes	2+	4+
9	yes	< 1	2+
10	yes	2+	4+
11	yes*	1+	2+
12	yes*	< 1	2+
13	yes*	< 1	3+
14	no	< 1	< 1
15	no	2+	2+

*Naturally-occurring challenge during outbreak
+Ratings determined on a scale of 0 to 4+ employing a stool filtrate as a source of antigen
#3 to 6 weeks after illness

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ANTIGENIC RELATEDNESS OF SMA, NORWALK AND HAWAII AGENTS.

The analysis of serum antibody responses to the Norwalk, Hawaii and Snow Mountain agents as determined by immune electron microscopy is presented in Table 2 (sera was available from previous studies). Significant antibody rises to the homologous antigens are demonstrated with all three agents. No heterologous rises are seen among any of the agents. This suggests that the three agents are antigenically distinct as determined by this technique.

Table 2. ANALYSIS OF SERUM ANTIBODY RESPONSES TO NORWALK, HAWAII, AND SNOW MOUNTAIN AGENTS*(SMA) BY IMMUNE ELECTRON MICROSCOPY (IEM)

Agent which induced illness in challenge study	Rating of serum specimens to homologous and heterologous antigens					
	Norwalk Antigen		Hawaii Antigen		SMA Antigen	
	pre	post	pre	post	pre	post
Norwalk (1)	1+	4+	2+	2+	< 1	< 1
Norwalk (2)	1+	3+	2+	1-2+	1+	1+
Hawaii (1)	2+	2+	1+	3+	1+	1+
Hawaii (2)	1+	1+	1+	3+	2+	2+
SMA (1)	2+	2+	1+	1+	< 1	3+
SMA (2)	1+	1+	2+	2+	2+	4+

*Challenge studies performed previously

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RADIOIMMUNOASSAY FOR NORWALK AGENT.

A highly sensitive and specific solid phase radioimmunoassay was established for detection of both Norwalk antigen and antibody to the Norwalk agent. This assay, which had previously been established by workers at the NIH (9), was used as a model system to develop a similar assay for SMA. Reagents from previously performed studies were used. Representative results for the assay for antigen are presented in Table 3. Antigen was detected only in stools post challenge, and was seen at peak titer generally 24 to 48 hours after the onset of illness. The assay appeared to be highly specific for the Norwalk agent as reflected by the lack of cross-reaction with Hawaii and Snow Mountain agents, with known parvoviruses, with enteroviruses, and with feline calicivirus as previously reported (9).

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TABLE 3. RADIOIMMUNOASSAY FOR NORWALK ANTIGEN IN STOOL FILTRATES FROM VOLUNTEER CHALLENGED WITH NORWALK AGENT

Stool Filtrate		P/N*
Pre-challenge stool filtrate		1.0
24 hrs post-challenge stool filtrate		2.2+
48 hrs post-challenge stool filtrate		4.1+
72 hrs post-challenge stool filtrate		1.7
96 hrs post-challenge stool filtrate		1.2
Norwalk infectious inoculum:	undiluted	4.6+
	1:10	3.8+
	1:100	2.2+
	1:1000	1.2
	1:10000	1.1
SMA infectious inoculum:	undiluted	1.1

*P/N ratio determined by solid phase radio immunoassay
+P/N $\geq$ 2 indicates presence of Norwalk antigen

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Antibody titers as determined by radioimmunoassay to the Norwalk agent are presented in Table 4. Significant antibody rises were noted in volunteers challenged with the Norwalk agent, but not in those challenged with Hawaii or Snow Mountain agents. This test also appears to be highly sensitive and specific for detection of anti-Norwalk antibody.

TABLE 4. ANTIBODY TITERS TO NORWALK AGENT IN SERA OF VOLUNTEERS CHALLENGED WITH NORWALK, HAWAII, AND SNOW MOUNTAIN AGENTS AS DETERMINED BY RADIOIMMUNOASSAY

Agent which induced illnes	pre-challenge serum	post-challange serum
Norwalk (1)	< 100*	> 12,800
Norwalk (2)	< 200	6,400
Norwalk (3)	< 100	6,400
Norwalk (4)	< 100	12,800
Hawaii (1)	400	400
Hawaii (2)	< 100	< 100
SMA (1)	200	200
SMA (2)	< 100	< 100

*Titer as determined by RIA blocking test for antibody to Norwak antigen

+Serum obtained 3 to 6 weeks after illness

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DEVELOPMENT OF A RADIOIMMUNOASSAY FOR THE SNOW MOUNTAIN AGENT (SMA).

Because the technical difficulty in performing immune electron microscopy necessarily limits the number of specimens which can be examined, the major thrust of the research effort was in the development of a radioimmunoassay for the Snow Mountain Agent. With the identification of serum specimens which had marked differences in antibody ratings by IEM, along with the identification and partial purification of the Snow Mountain Agent in stool filtrates, a sensitive radioimmunoassay for the Snow Mountain Agent was developed. Results of the radioimmunoassay for SMA antigen in stool filtrates from the three volunteers challenged with SMA in whom antigen could be detected in stools are presented in Tables 5, 6, and 7. The pattern of shedding of SMA antigen was similar to that previously observed for Norwalk antigen. SMA antigen was generally detected 24 to 52 hours after challenge and was no longer detectable beyond five days after challenge. Antigen was not detected in stools prior to challenge, nor in stools from volunteers who did not become ill. The radioimmunoassay did not cross-react with stools which contained Norwalk agent or the Hawaii agent. SMA antigen was detected in 11 separate stool specimens from the previously described volunteer studies. RIA antigen was always detected in stools in which virus particles were seen by immune electron microscopy, as well as in a number of IEM negative stools. The latter observation suggests that the RIA is more sensitive than IEM or that alternatively, RIA may detect soluble as well as virus-associated antigen. Additional studies are required to clarify this question. Therefore, the RIA which has been established for SMA antigen appears to be highly sensitive and specific.

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TABLE 5. RADIOIMMUNOASSAY FOR SMA ANTIGEN IN STOOL FILTRATES FROM VOLUNTEER #5 CHALLENGED WITH SMA

Time at which stool was passed	P/N*
24 hrs pre-challenge	0.80
6 hrs post-challenge	0.82
13 hrs post-challenge	1.01
24 hrs post-challenge	2.01+
120 hrs post-challenge	0.83

*P/N ratio determined by solid phase radioimmunoassay

+P/N $\geq$ 2 indicates the presence of SMA Antigen

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TABLE 6. RADIOIMMUNOASSAY FOR SMA ANTIGEN IN STOOL FILTRATES FROM VOLUNTEER #9 CHALLENGE WITH SMA

Time at which stool was passed	P/N*
24 hrs pre-challenge	0.90
4 hrs post-challenge	0.93
30 hrs post-challenge	1.47
50 hrs post-challenge	1.51
52 hrs post-challenge	3.17+
70 hrs post-challenge	3.79
72 hrs post-challenge	2.53
77 hrs post-challenge	3.61
96 hrs post-challenge	1.10
122 hrs post-challenge	1.19

*P/N ratio determined by solid phase radioimmunoassay

P/N $\geq$ 2 indicates the presence of SMA antigen

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TABLE 7. RADIOIMMUNOASSAY FOR SMA ANTIGEN IN STOOL FILTRATES FROM VOLUNTEER #10 CHALLENGED WITH SMA

Time at which stool was passed	P/N*
48 hrs pre-challenge	1.05
24 hrs pre-challenge	0.90
6 hrs post-challenge	0.96
24 hrs post-challenge	0.71
31 hrs post-challenge	2.29+
34 hrs post-challenge	5.26
52 hrs post-challenge	12.69
72 hrs post-challenge	10.57
96 hrs post-challenge	6.66
120 hrs post-challenge	4.05
144 hrs post-challenge	1.32
164 hrs post-challenge	1.20

*P/N ratio determined by solid phase radioimmunoassay
+P/N $\geq$ 2 indicates the presence of SMA antigen

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RADIOIMMUNOASSAY FOR ANTIBODY TO SNOW MOUNTAIN AGENT (SMA).

Employing the above stool filtrates as a source of antigen, an RIA for antibody to SMA was developed. The results of RIA antibody determinations are presented in Table 8. Serum antibody rises were observed in 8 of 9 volunteers with experimentally-induced illness after challenge with SMA, and in three of three naturally-occurring cases. No antibody rises were detected in three volunteers who were challenged but did not become ill. Serum antibody rises correlated well with those obtained by immune electron microscopy. Thus, the RIA for antibody also appears to be a highly sensitive and specific assay.

TABLE 8. RADIOIMMUNOASSAY FOR ANTIBODY TO SNOW MOUNTAIN AGENT (SMA)

Subject	Illness following challenge with SMA	Dilution of SMA inoculum	Pre Challenge Serum antibody titer	Post Challenge Serum antibody titer
1	yes	10^0	100	6400
2	yes	10^0	200	800
3	yes	10^0	100	800
4	no	10^0	3200	1600
5	yes	10^0	100	1600
6	yes	10^0	< 100	200
7	yes	10^{-1}	200	200
8	yes	10^{-1}	< 100	1600
9	yes	10^{-2}	< 100	800
10	yes	10^{-2}	100	3200
11*	yes	-	400	1600
12*	yes	-	100	800
13*	yes	-	100	1600
14	no	10^{-3}	100	200
15	no	10^{-3}	200	200

*Naturally-occurring illness during the original Snow Mountain outbreak - acute and convalescent serum specimens are compared

ATTEMPTS TO DEVELOP IN VITRO CULTIVATION TECHNIQUES FOR SNOW MOUNTAIN AGENT.

Relatively few studies of cultivation of SMA in vitro have been performed during the project period because of the lack of a sensitive and convenient assay system. Early attempts at blind passage through standard tissue culture systems and human mononuclear cells as assayed by immune electron microscopy have been negative. Tissue cultures which have been employed include human embryonic kidney cells, low passage human fibroblasts, and human hepatocyte cultures. With the availability of the radioimmunoassay for SMA, an assay system is at hand with which intensive studies of in vitro cultivation systems could be carried out in a manner which would conserve the limited amount of reagents (stool filtrates and sera) available for in vitro investigation.

DICUSSION.

The studies carried out during the project period have clearly identified a new waterborne viral agent of gastroenteritis, the Snow Mountain Agent (SMA). This agent is present in the stools of naturally-occurring cases in the Snow Mountain outbreak, and has induced acute gastroenteritis after oral administration to normal volunteers in the form of 2% stool filtrates. Additional evidence which supports the etiologic significance of the virus particle are serum antibody rises detected in naturally-occurring and experimentally-induced cases of gastroenteritis, and the absence of such rises in individuals who were challenged and did not become ill. In addition, the virus is shed during acute illness, but is not detected prior to challenge or when illness is not present. The particle is 27 to 32 nm in diameter, has cubic symmetry, and morpho-

logically resembles the Norwalk and Hawaii agents. However, SMA appears to be antigenically distinct from both the Norwalk and Hawaii agents by immune electron microscopy as well as by radioimmunoassay.

In vitro detection of SMA was accomplished despite the inability to cultivate the agent in conventional tissue culture systems. Initially, virus particles were detected in stool specimens from the naturally-occurring outbreak employing immune electron microscopy. This cumbersome although powerful technique, relies on the ability of virus particles to be aggregated by specific antibody into a distinct cluster which can be readily recognized under the electron microscope. Such particles were observed in two of five stool specimens when pooled human gamma globulin was used as a source of antibody. The etiologic significance of such particles, however, remained uncertain until specific antibody rises in acute and convalescent serum specimens were demonstrated. To provide the reagents required for such studies, a series of volunteer experiments had been carried out previously in which stool filtrates containing the above virus particles were administered to normal volunteers with the subsequent induction of acute gastroenteritis. Employing convalescent serum from one such volunteer, virus-like particles were detected in stools from three additional volunteers who were acutely ill. After preliminary concentration and partial purification of these virus particles, immune electron microscopy tests were carried out in which serum antibody rises were demonstrated in individuals who were acutely ill with SMA-induced disease. This technique, although critical for the initial studies, is sufficiently cumbersome so that only a relatively small number of specimens could be examined.

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Because of this limitation, the major effort during the project period was expended upon the development of a solid phase radioimmunoassay for the Snow Mountain Agent. Despite intensive examination of combinations of stools and sera generated in the above studies, such an assay proved difficult to establish. The reason for these difficulties are unclear, but are likely related to the high degree of specificity required of the reagents in this technique. Thus, most anti-sera are not effective "traps or coating antibodies" in this assay, despite the fact that they have high titers of antibody against the agent. Development of this assay is a laborious trial and error procedure, which requires finding the proper combination of trapping antibody and radiolabeled antibody, with sufficient avidity and specificity. To illustrate the difficulty of this procedure, of 72 pairs of sera available to us from Norwalk infections, only 3 are suitable as reagents for the radioimmunoassay. To ascertain that the appropriate methodology was being employed, we undertook to establish a similar RIA for the Norwalk agent, which had been successfully studied in this manner. The establishment of our RIA for Norwalk, described above, was instrumental in the success of the subsequent studies of SMA. In these investigations, emphasis was placed upon those stools and sera which appeared to have high concentrations of antigen and antibody by immune electron microscopy. These studies led to the establishment of a highly specific and sensitive RIA for SMA antigen as well as for anti-SMA antibody. This assay detected SMA antigen in the stools of volunteers following challenge with infectious inoculum, and demonstrated a shedding pattern similar to that previously demonstrated with the Norwalk agent. Serum antibody responses were also demonstrated in both

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experimentally-induced and naturally-occurring illness, and correlated well with antibody responses as determined by immune electron microscopy. While the RIA appeared to be equally sensitive to IEM in the detection of serum antibody rises, RIA was significantly more sensitive in the detection of antigen in stool than IEM. The latter phenomenon may reflect either an increased sensitivity of the radioimmunoassay for virus particles, or alternatively, the detection of soluble (non-virus associated) antigen which is not detected by IEM. Additional studies are required to resolve this question.

The establishment of the RIA for SMA now provides an enormously powerful tool with which to pursue studies of that agent. Clearly a major requirement for the field would be the establishment of a suitable *in vitro* culture system with which to detect and study SMA as well as other Norwalk-like agents. Because a large number of samples can now be analyzed with relatively small amounts of reagents, intensive investigation of promising *in vitro* culture systems can be carried out. Although the limited number of *in vitro* cultivation studies which were carried out during the project period appeared to be negative, extensive studies can now be efficiently pursued. Similarly, the epidemiologic impact of disease induced by this agent can now be evaluated in studies of both acute outbreaks and seroprevalence. These studies are particularly important because the effect of waterborne spread of SMA was multiplied many fold by the rapid person-to-person transmission which also took place in the outbreak. Because of this explosive epidemic pattern, environmental sampling for this agent should be carried out, along with an evaluation of procedures for decontamination of drinking water.

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REFERENCES

1. National Center for Health Statistics, U.S. Department of Health, Education and Welfare. Current estimates from the Health Interview Survey, United States - 1972 (1973). Vital and Health Statistics, Publication No. (HRA) 74-1512, Series 10, No. 85.

2. Ryder, R.W., Sack, D.A., Kapikian, A.Z., et al. Enterotoxigenic Escherichia coli and reovirus-like agent in rural Bangladesh. Lancet i: 659, 1976.

3. Greenberg, H.B., Valdesuso, J., Yolken, R.H., et al.: Role of Norwalk agent in outbreaks of nonbacterial gastroenteritis. J. Inf. Dis. 139: 564, 1979.

4. Morens, D.A., Zwieghaft, R.M., Vernon, T.M., Gary, W.G., Eslien, J. J. Wood, B.T., Holman, R.C., and Dolin, R.: A waterbone outbreak of gastroenteritis with secondary person to person spread: association with a viral agent. Lancet i: 964, 1979.

5. Adler, J.L. and Zickl, R.: Winter Vomiting disease. J. Inf. Dis 119: 668, 1969.

6. Center for Disease Control: Water-related disease outbreaks - Annual Summary 1978, HHS publication NO (CDC) 80-8385, U.S. Government Printing Office, pp. 1-26, 1980.

7. Murphy, A.H., Grohmann, G.S., Christopher, P.J., Lopez, W.A., Davey, G.R., Millsom, R.H.: An Australia-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. Med. J. Aus. 2: 329, 1079.

8. Knight, V.: The use of volunteers in medical virology. Prog. Med. Virol. 6: 1964.

9. Greenberg, H.B., Wyatt, R.G., Valdesuso, J., Kalica, A.R., London, W.T., Chanock, R.M., and Kapikian, A.Z.: Solid-phase radioimmunoassay for detection of the Norwalk strain of acute nonbacterial epidemic gastroenteritis virus and its antibodies. J. Med. Virol. 2: 97, 1978.

10. Dolin, R., Reichman, R.C., and Fauci, A.S.: Lymphocyte populations in acute viral gastroenteritis. Inf. and Imm. 14: 422-428, 1976.

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